ISSN: 0001-5113 AADRAY	ACTA ADRIAT., 61 (2): 135 - 144, 2020	ORIGINAL SCIENTIFIC PAPER

First evidence of domoic acid production in *Pseudo-nitzschia calliantha* cultures from the central Adriatic Sea

Jasna ARAPOV[1], Ivana UJEVIĆ[1], Maja STRAKA[1], Sanda SKEJIĆ[1*], Mia BUŽANČIĆ[1], Ana BAKRAČ[1] and Živana NINČEVIĆ GLADAN[1]

1Institute of Oceanography and Fisheries, Šetalište Ivana Meštrovića 63, 21 000 Split, CROATIA

****Corresponding author: sanda@izor.hr***

In this study, three isolates of the potentially toxic diatom genus Pseudo-nitzschia *were analysed for morphological and toxicological features. Cultures of* Pseudo-nitzschia *were established from seawater samples collected from the southern part of the Velebit Channel (central Adriatic Sea) during February 2019. All culture isolates were identified by scanning electron microscopy (SEM) as* Pseudo-nitzschia calliantha*. Domoic acid (DA) production was confirmed in all isolates analysed. The highest concentrations of cellular DA were found in early culture stages, with the lowest cell abundance, for all* P. calliantha *isolates. This study is the first to report DA production by* P. calliantha *isolated from the Adriatic Sea.*

Key words: *Pseudo-nitzschia*, morphology, toxicity, domoic acid

INTRODUCTION

Diatoms of the genus *Pseudo-nitzschia* are common constituents of the marine phytoplankton community and regularly occur throughout the world's oceans (LELONG *et al*., 2012; BATES *et al*., 2018), including the Adriatic Sea (VILIČIĆ *et al*., 2007; BUŽANČIĆ *et al*., 2012; MARIĆ *et al*., 2012; SKEJIĆ *et al*., 2014; NINČEVIĆ GLADAN *et al*., 2020). Due to its ability to produce the neurotoxin domoic acid (DA), *Pseudo-nitzschia* has gained considerable scientific attention. With the development of new molecular tools, research of *Pseudo-nitzschia* resulted in the discovery of many new species. LELONG *et al*. (2012) reported 37 *Pseudo-nitzschia* species, of which, 14 were toxic. To date, this genus comprises 60 described species, among which, 26 are confirmed toxic (BATES *et al*., 2018; HUANG *et al*., 2019; LUNDHOLM, 2019). Laboratory studies have shown that toxin production by *Pseudo-nitzschia* species is complex because species toxicity is influenced by abiotic (silicates or phosphates limitation, inorganic or organic nitrogen forms, temperature, salinity, irradiance) and biotic (bacteria, zooplankton) factors, as well as interactions between two or more factors (reviewed in LELONG *et al*., 2012; TRAINER *et al*., 2012; BATES *et al*., 2018).

Along the Croatian coast, the diversity of the *Pseudo-nitzschia* genus is based on the descriptions of 10 species (LJUBEŠIĆ *et al*., 2011; MARIĆ PFANNKUCHEN, 2013; ARAPOV *et al*., 2017; ARAPOV *et al*., 2019). The toxicity of these species has

not been reported, although low levels of DA were determined sporadically in shellfish from the eastern Adriatic coast (UJEVIĆ *et al*., 2010; LJUBEŠIĆ *et al*., 2011; ARAPOV *et al*., 2016; ARAPOV *et al*., 2017; UJEVIĆ *et al*., 2019). This study presents the first evidence of DA production in cultures of *Pseudo-nitzschia* species, in particular *P. calliantha*, in isolates from the eastern Adriatic coast.

MATERIAL AND METHODS

Cell isolation and SEM analysis

Pseudo-nitzschia calliantha strains were isolated from a phytoplankton net sample (20 µm pore size), collected at station M-S1 (44.2696°N, 15.51655°E) in February 2019 (Fig. 1). The net was towed between the surface and a depth of 7 m. Single-cell or chain was isolated under an inverted light microscope (Leica DMI4000B) using a sterile glass micropipette and transferred to 1 mL of f/2 medium in a 24-well tissue culturing plate. The plate was kept at 18 ± 0.5 °C, with a photoperiod of 12:12 h (light:dark) at 108 µmol photons m^{-2} s^{-1} for 7 days. Afterwards, the isolates were transferred to culturing flasks containing 30 mL of f/2 medium to increase density (initial flask). For morphological and toxicological analyses, three *Pseudo-nitzschia calliantha* cultures were successfully established: isolates D4, D5, and E2.

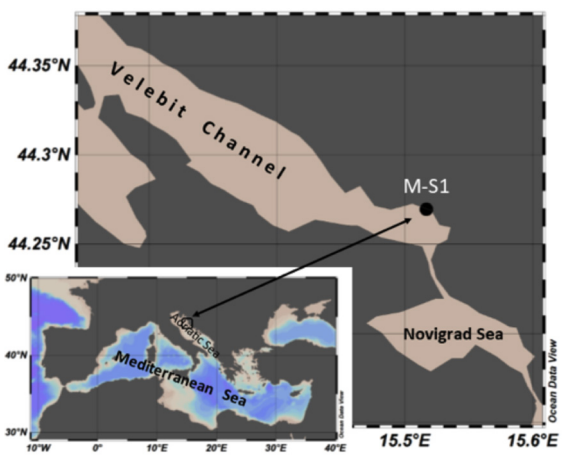

Fig. 1. Southern part of the Velebit Channel (central Adriatic Sea) showing sampling station M-S1 (Modrič-Seline)

Morphological characteristics were examined using an SEM (Tescan, MIRA3). Subsamples of 10 mL each were taken from the initial flask and fixed with Lugol solution. *Pseudo-nitzschia* frustules were cleaned according to the method described in HASLE & FRYXELL (1970), filtered on polycarbonate membrane filters (pore size 1 µm, Nucleopore, Whatman), and dried in a desiccator for a minimum of 24 h. After that, filters were gold coated and analysed with the SEM.

Toxin analyses

For toxin analyses, 2 mL of each *Pseudo-nitzschia* isolate from the initial flask were inoculated into three flasks containing 80 mL of f/2 medium. To analyse the toxin content at different stages of culture, subsamples of 3 mL were collected on day 6 and day 13 for all isolates. Depending on cell density, additional subsamples were taken on days 22, 27, and 36 for isolates E2, D5, and D4, respectively. Subsamples of cultures were fixed with Lugol solution and cell abundance was analysed with an inverted light microscope (Leica DMI4000B) in a Sedgewick Rafter counting chamber.

At least 50 fields were counted per subsample, and empty frustules were not included in the count. On sampling days, the whole culture was filtered through GF/F filters (Whatman, pore size 0.7 µm) and frozen at -20 °C until toxin analysis. The exact volume of each filtered culture was measured, to calculate the total number of cells in the whole culture, and analysed for toxin content. At the time of analysis, filters were thawed and sonicated for 1 min in 5 mL of 100% MeOH, centrifuged at 2000 g for 10 min and filtered through 0.22 µm filters (FilterBio, Naylon Syringe Filter, 13 mm diameter).

The filtered methanol extracts of *Pseudo-nitzschia* species were analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS, Agilent Technologies) to determine DA content. The tandem mass spectrometer was equipped with a Triple Quad 6410, Degasser 1200, Quaternary Pump 1200, Auto sampler 1290, and Thermostatted Column Compartment 1290. Chromatograph conditions for

the Poroshell 120 (EC-C18, 2.1 mm x 50 mm, 2.7 µm) column coupled to the Poroshell 120 (EC-C18, 2.1 mm x 5 mm, 2.7 µm) pre-column were: flow 0.3 mL/min, temperature 30 °C, and mobile phase B gradient from 10 to 80% in 4 min, held for 2 min, and recovered to initial condition for 5 min. Mobile phase A consisted of 100% water with 2 mM ammonium formate and 50 mL formic acid, while mobile phase B consisted of 95% acetonitrile, 5% water with 2 mM ammonium formate, and 50 mM formic acid. Quantification of DA by the multiple reaction monitoring mode (MRM) were performed in positive ion mode. Electrospray ionisation (ESI) was applied as the optimum ion source interface for DA. The identification of DA was based on the retention time of DA in HPLC and the exact of protonated parent ion (312.2 m/z) and the most intense product ion (266.1 m/z). For qualitative identification, a second selected fragment (248.0 m/z) based on intensity is required.

Quantification of DA was performed using the calibration curves of six working standard solutions. The working standard solutions were prepared by diluting stock solutions containing a mixture of certified standards (DTXs, PTX-2, AZAs, SPX, GYM, and DA, National Research Council of Canada, Halifax, Canada). The concentration DA stock solution was 3000 ng mL^{-1} and prepared in methanol. Six DA calibration working solutions were prepared in concentrations ranging between 30 and 450 ng mL^{-1}. The cellular DA concentration (ng DA $cell^{-1}$) was calculated by multiplying the DA concentration in the whole culture (ng mL^{-1}) with a volume of extraction solvent (5 mL) and dividing by the total cell abundance in the whole culture.

RESULTS AND DISCUSSION

Three clonal cultures were successfully established for morphological and toxicological analyses. Morphologically, cultures isolated from the Velebit Channel were confirmed as *Pseudo-nitzschia calliantha* (Fig. 2). For each isolate, 10 valves were measured, and morphological characteristics were observed by SEM and presented in Table 1. In the valve view, cells were linear and symmetrical (Fig. 2A). The transapical and apical axes of cultured cells were within the range 1.61–2.17 µm and 67.73–90.90 µm, respectively. A central interspace occupied 3.5 to 6 striae with central nodules (Fig. 2B). There were 17 to 21 fibulae and 32 to 37 interstriae per 10 µm, while the number of sectors within the poroids varied from 3 to 12. Sectors within poroids were arranged in a circle with 33–47% of poroids having a central sector and thus exhibiting the characteristic flower pattern (Fig. 2B). The structure of the girdle bands showed that the first band, the valvocopula, had 43 to 45 striae per 10µm. Each band stria was two to three poroids wide but varied in height. Valvocopula were 4 to 6 poroids high, while the second and third bands had arrangements of 3–4 and 2–3 poroids high, respectively (Fig. 2D). In comparison, cells from three established *P. calliantha* cultures showed similar morphological characteristics. Slight differences were observed for *P. calliantha* isolate D4, which had longer transapical axes, shorter apical axes, and fewer number of sectors within poroids than of isolates D5 and E2. The morphological characteristics of cultured *P. calliantha* (D4, D5, and E2) cells correspond to the original description by LUNDHOLM *et al.* (2003) and those previously observed from the Adriatic Sea (BURIĆ *et al*., 2008; LJUBEŠIĆ *et al*., 2011; MARIĆ *et al*., 2011; ARAPOV *et al*., 2016, 2017; TURK DERMASTIA *et al*., 2020) and the Mediterranean Sea (SAHRAOUI *et al*., 2009; MOSCHANDROU & NIKOLAIDIS, 2010; QUIJANO-SCHEGGIA *et al*., 2010). Identification of *P. calliantha* in this study was not confirmed by molecular tools; however, it shows good correspondence with morphological descriptions of previous findings (references herein) and it is commonly found in the eastern Adriatic.

Toxin analyses confirmed DA production by all three *P. calliantha* isolates analysed in this study. Cellular DA values ranged between 0.0022 and 0.0351 pg $cell^{-1}$ for the D4 isolate, 0.0032 and 0.0855 pg $cell^{-1}$ for D5, and 0.0038 and 0.0058 pg $cell^{-1}$ for E2 (Table 2). The highest concentrations of cellular DA were found in the early stages of development, with the lowest cell abundance, for all *P. calliantha* isolates.

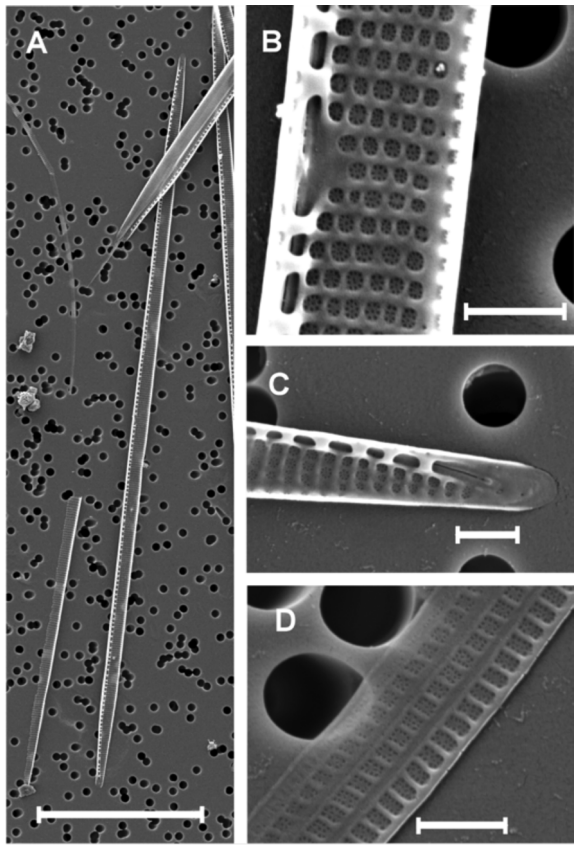

Fig. 2. Morphology of P. calliantha *E2 isolate observed by scanning electron microscopy: A) whole cell in valve view, B) central part of valve showing central interspace with central nodule and poroid structure, C) apical part of valve, and D) structure of singular bands. Scale bars represent A) 20 µm and B–D) 1 µm*

Comparing the *P. calliantha* isolates analysed in this study, the highest DA content per cell was found in all stages of the D5 culture.

The species *P. calliantha* is a globally distributed *Pseudo-nitzschia* species, and although its toxicity has been reported from different areas, more studies cite non-toxic strains of *P. calliantha* (TRAINER *et al*., 2012; BATES *et al*., 2018 and references therein). Cellular DA content is reported within the range from 0.054 fg cell^{-1} (WADT *et al*., 2017) to 0.95 pg cell^{-1} (BESIKTEPE *et al*., 2008). Given the results of the toxin analyses, measured DA concentrations for *P. calliantha* isolates from the central Adriatic Sea were lower than those reported by LUNDHOLM *et al*. (1997) and cited in LUNDHOLM *et al*. (2003) at 0.221 pg cell^{-1}, BESIKTEPE *et al*. (2008) at 0.95 pg cell^{-1}, and STONIK *et al*. (2019) at 0.441 pg cell^{-1}, but consistent with values reported by ALVAREZ *et al*. (2009) at 0.01 pg cell^{-1}, THESSEN *et al*. (2009) at 0.0018–0.0057 pg cell^{-1}, and STONIK *et al*. (2019) at 0.015–0.077 pg cell^{-1}.

Consistent with our findings, BESIKTEPE *et al*. (2008) recorded higher cellular DA levels during the early exponential phase of development, as reported for *P. cuspidata* (AURO & COCHLAN, 2013). In contrast, many studies confirmed higher DA production in the stationary phase, suggesting that the mechanism of DA production may differ among species and strains (BATES *et al*., 2018).

Although the presence of 15 potentially toxic *Pseudo-nitzschia* species were recorded in the Mediterranean Sea (ZINGONE *et al*., 2020), DA production was only confirmed for 7 species. The only two species from the Adriatic Sea confirmed to produce DA are *P. multistriata* (PISTOCCHI *et al*., 2012) and *P. delicatissima* (PENNA *et al*., 2013). In other parts of the Mediterranean, toxicity was recorded for *P. brasiliana*, *P. calliantha*, and *P*. cf. *delicatissima* from Bizerte Lagoon (south-west Mediterranean, SAHRAOUI *et al*., 2009, 2011); *P. galaxiae*, *P. pseudodelicatissima*, and *P. pungens* var. *pungens* from Greek coastal waters (north-east Mediterranean, MOSCHANDREOU *et al*., 2010, 2012), and for *P. galaxiae* and *P. multistriata* from the Tyrrhenian Sea (ORSINI *et al*., 2002; CERINO *et al*., 2005; AMATO *et al*., 2010).

To our knowledge, this study is the first report of DA production by *Pseudo-nitzschia calliantha* isolated from the Adriatic Sea. *Pseudo-nitzschia* species frequently occur in the Adriatic phytoplankton community with increasing abundance over the last 15 years (NINČEVIĆ GLADAN *et al*., 2020) and are occurring in all seasons (TURK DERMASTIA *et al*., 2020). Our results present new insight into the toxicity of this important diatom genus, which is still mostly unexplored along the eastern Adriatic coast.

ACKNOWLEDGEMENTS

We would like to thank Mr. Nikša NAZLIĆ for conducting field sampling. This study was fully funded by the Croatian Science Foundation

Table 1. Morphological characteristics of three cultured strains of P. calliantha *(isolates D4, D5, and E2). Number of measured cells was 10; minimum and maximum values are given in bold; average ± standard deviation values are specified row below. Number of measured poroids (n) is presented in parentheses*

Species (n)	Width (µm)	Length (µm)	CN	Fibulae (10µm)	Interstriae (10µm)	Poroid rows	Poroids (1µm)	Sectors within poroid (n)	Band striae (10µm)	Structure of valvocopula (width x height)
P. calliantha D4	**1.72-2.17**	**67.73-83.63**	4-6	**17-20**	**32-37**	1	**4-5**	**3-10**	**43-45**	**2-3 x 4-5**
	1.92±0.13	80.23±4.69		18.70±1.06	34.70±1.83			(57)	44.10±0.57	
P. calliantha D5	**1.61-1.95**	**87.13-90.90**	3.5-6	**17-20**	**34-37**	1	**4-5**	**3-11**	**44-45**	**2-3 x 4-6**
	1.78±0.13	88.41±1.21		18.60±0.97	35.50±1.08			(72)	44.33±0.51	
P. calliantha E2	**1.56-1.80** 1.73±0.07	**85.18-87.52** 86.26±0.75	4-5	**17-21** 18.85±1.29	**34-37** 35.89±0.88	1	**3.5-6**	**3-12** (68)	**44-45** 44.30±0.48	**2-3 x 4-6**

Table 2. Concentration of domoic acid (DA) determined by LC-MS/MS in P. calliantha *cultured strains (D4, D5, E2) at different cell abundances*

Species	Culture age (days)	Cell density in culture (st mL^{-1})	Cell abundance in whole culture	Average DA concentration in culture (ng mL^{-1})± SD	DA concentration per cell (pg $cell^{-1}$)
P. calliantha D4	6	4840	266200	1.8671±0.9775	0.0351
	13	52520	3098680	1.3431±0.3248	0.0022
	36	138200	7255500	< LOD	/
P. calliantha D5	6	2720	131920	2.2557±0.4433	0.0855
	13	25800	1332820	2.2651±0.8495	0.0085
	27	75060	3640410	2.3024±0.1343	0.0032
P. calliantha E2	6	6180	352260	0.5129±0.0762	0.0058
	13	16180	857540	0.6472±0.1179	0.0038
	22	16920	820620	< LOD	/

under the project IP-2014-09-3606 "Marine plankton as a tool for assessment of climate and anthropogenic influence on the marine ecosystem, MARIPLAN".

REFERENCES

ÁLVAREZ, G., E. URIBE, S. QUIJANO-SCHEGGIA, A. LÓPEZ-RIVIERA, C. MARIÑO & J. BLANCO. 2009. Domoic acid production by *Pseudo-nitzschia australis* and *Pseudo-nitzschia calliantha* isolated from North Chile. Harmful Algae, 8: 938–945.

AMATO, A., A. LÜDEKING & W.H.C.F. KOOISTRA. 2010. Intracellular domoic acid production in *Pseudo-nitzschia multistriata* isolated from the Gulf of Naples (Tyrrhenian Sea, Italy). Toxicon, 55: 157-161.

ARAPOV, J., I. UJEVIĆ, D. MARIĆ PFANNKUCHEN, J. GODRIJAN, A. BAKRAČ, Ž. NINČEVIĆ GLADAN & I. MARASOVIĆ. 2016. Domoic acid in phytoplankton net samples and shellfish from the Krka River estuary in the Central Adriatic Sea. Mediterranean Marine Science, 17: 340–350.

ARAPOV, J., S. SKEJIĆ, M. BUŽANČIĆ, A. BAKRAČ, O. VIDJAK, N. BOJANIĆ, I. UJEVIĆ & Ž. NINČEVIĆ GLADAN. 2017. Taxonomical diversity of *Pseudo-nitzschia* from the Central Adriatic Sea. Phycological Research, 65: 280–290.

ARAPOV, J., M. BUŽANČIĆ, A. PENNA, S. CASABIANCA, S. CAPELLACCI, F. ANDREONI, S. SKEJIĆ, A. BAKRAČ, M. STRAKA & Ž. NINČEVIĆ GLADAN. 2019. Late summer bloom of *Pseudo - nitzschia* species: morphological and molecular characterisation. In: Seventh European Phycological Congress, Zagreb, Croatia, 25-30 August 2019. Taylor & Francis, pp. 213–213.

AURO, M. E. & W. P. COCHLAN. 2013. Nitrogen utilization and toxin production by two diatoms of the *Pseudo-nitzschia pseudodelicatissima* complex: *P. cuspidata* and *P. fryxelliana*. Journal of Phycology, 49: 156-169.

BATES, S. S., K. A. HUBBARD, N. LUNDHOLM, M. MONTRESOR & C. P. LEAW. 2018. *Pseudo-nitzschia*, *Nitzschia*, and domoic acid: New research since 2011. Harmful Algae, 79: 3–43.

BESIKTEPE, S., L. RYABUSHKO, D. EDIGER, D. YILMAZ, A. ZENGINER, V. RYABUSHKO & R. LEE. 2008. Domoic acid production by *Pseudo-nitzschia calliantha* Lundholm, Moestrup et Hasle (bacillariophyta) isolated from the Black Sea. Harmful Algae, 7: 438–442.

BURIĆ, Z., D. VILIČIĆ, K. CAPUT MIHALIĆ, M. CARIĆ, K. KRALJ & N. LJUBEŠIĆ. 2008. *Pseudo-nitzschia* blooms in the Zrmanja River estuary (eastern Adriatic Sea). Diatom Research, 23: 51–63.

BUŽANČIĆ, M., Ž. NINČEVIĆ GLADAN, I. MARASOVIĆ, G. KUŠPILIĆ, B. GRBEC & S. MATIJEVIĆ. 2012. Population structure and abundance of phytoplankton in three bays on the eastern Adriatic coast: Šibenik Bay, Kaštela Bay and Mali Ston Bay. Acta Adriat., 53: 413-435.

CERINO, F., L. ORSINI, D. SARNO, C. DELL'AVERSANO, L. TARTAGLIONE & A. ZINGONE. 2005. The alternation of different morphotypes in the seasonal cycle of the toxic diatom *Pseudo-nitzschia galaxiae*. Harmful Algae, 4: 33-48

HASLE, G. R. & G. A. FRYXELL. 1970. Diatoms: Cleaning and mounting for light and electron microscopy. Transactions of the American Microscopical Society, 89: 469–474.

HUANG C. X., H. C. DONG, N. LUNDHOLM, S. T. TENG, G. C. ZHENG, Z. J. TAN, P. T. LIM & Y. LI. 2019. Species composition and toxicity of the genus *Pseudo-nitzschia* in Taiwan Strait, including *P. chiniana* sp. nov. and *P. qiana* sp. nov. Harmful Algae, 84: 195–209.

LELONG, A., H. HÉGARET, P. SOUDANT & S. S. BATES. 2012. *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. Phycologia, 51: 168–216.

LUNDHOLM, N. 2019. Bacillariophyceae. In: IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (N. Lundholm (Editor)). Available online at http: //www.marinespecies.org/hab. Accessed on 2019-09-24

LUNDHOLM, N., J. SKOV, R. POCKLINGTON & Ø. MOESTRUP. 1997. Studies on the marine planktonic diatom *Pseudo-nitzschia*. 2. Autoecology of *P. pseudodelicatissima* base on

isolates from Danish coastal waters. Phycologia, 36: 381-388.

LUNDHOLM, N., Ø. MOESTRUP, G. R. HASLE & K. HOEF-EMDEN. 2003. A study of the *Pseudo-nitzschia pseudodelicatissima*/*cuspidata* complex (Bacillariophyceae): what is *P. pseudodelicatissima*? Journal of Phycology, 39: 797–813.

LJUBEŠIĆ, Z., S. BOSAK, D. VILIČIĆ, K. KRALJ BOROJEVIĆ, D. MARIĆ J. GODRIJAN, I. UJEVIĆ, P. PEHAREC & T. ĐAKOVEC. 2011. Ecology and taxonomy of potentially toxic *Pseudo-nitzschia* species in Lim Bay (north-eastern Adriatic Sea). Harmful Algae, 10: 719–722.

MARIĆ PFANNKUCHEN, D. 2013. Potentially toxic diatom genus Pseudo-nitzschia in the northern Adriatic Sea: ecological, taxonomic and molecular features. Ph.D. dissertation, University of Zagreb, Zagreb, Croatia, 195 pp.

MARIĆ, D., Z. LJUBEŠIĆ, J. GODRIJAN, D. VILIČIĆ, I. UJEVIĆ & R. PRECALI. 2011. Blooms of the potentially toxic diatom *Pseudo-nitzschia calliantha* Lundholm, Moestrup & Hasle in coastal waters of the Northern Adriatic Sea. Estuarine, Coastal and Shelf Science, 92: 323–331.

MARIĆ, D., R. KRAUS, J. GODRIJAN, N. SUPIĆ, T. DJAKOVEC & R. PRECALI. 2012. Phytoplankton response to climatic and anthropogenic influences in the north-eastern Adriatic during the four last decades. Estuarine, Coastal Shelf Science, 115: 98-112.

MOSCHANDREOU, K. K. & G. NIKOLAIDIS. 2010. The genus *Pseudo-nitzschia* (Bacillariophyceae) in Greek coastal waters. Botanica Marina, 53: 159–172.

MOSCHANDREOU, K. K., A. D. BAXEVANIS, P. KATIKOU, D. PAPAEFTHIMIOU, G. NIKOLAIDIS & T. J. ABATZOPOULOS. 2012. Inter- and intraspecific diversity of *Pseudo-nitzschia* (Bacillariophyceae) in the northeastern Mediterranean. European Journal of Phycology, 47(3): 321-339.

NINČEVIĆ GLADAN, Ž., F. MATIĆ, J. ARAPOV, S. SKEJIĆ, M. BUŽANČIĆ, A. BAKRAČ, M. STRAKA, Q. DEKNEUDT, B. GRBEC, R. GARBER & N. NAZLIĆ. 2020. The relationship between toxic phytoplankton species occurence and environmental and meteorological factors along the Eastern Adriatic Coast. Harmful Algae, 92: doi.org/ 10.1016/j.hal.2020.101745.

ORSINI, L., D., SARNO, G. PROCACCINI, R. POLETTI, J. DAHLMANN & M. MONTRESOR. 2002. Toxic *Pseudo-nitzschia multistriata* (Bacillariophyceae) from the Gulf of Naples: morphology, toxin analysis and phylogenetos relatioships with other *Pseudo-nitzschia* species. European Journal of Phycology, 37(2): 247-257.

PENNA, A., S. CASABIANCA, F. PERINI, M. BASTIANINI, E. RICCARDI, S. PIGOZZI & M. SCARDI. 2012. Toxic *Pseudo-nitzschia* spp. in the northwestern Adriatic Sea: caharacterization od species composition by genetic and molecular quantitative analyses. Journal of Plankton Research, 35(2): 352-366.

PISTOCCHI, R., F. GUERRINI, L. PEZZOLESI, M. RICCARDI, S. VANUCCI, P. CIMINIELLO, C. DELL'AVERSANO, M. FORINO, E. FATTORUSSO, L. TARTAGLIONE, A. MILANDRI, M. POMPEI, M. CANGINI, S. PIGOZZI & E. RICCARDI. 2012. Toxin Levels and Profiles in Microalgae from the North-Western Adriatic Sea-15 Years of Studies on Cultured Species. Marine Drugs, 10: 140-162.

QUIJANO-SCHEGGIA, S., E. GARCÉS, K.B. ANDREE, P. IGLESIA, J. DIOGÈNE, J.-M. FORTUÑO & J. CAMP. 2010. *Pseudo-nitzschia* species on the Catalan coast: characterization and contribution to the current knowledge of the distribution of this genus in the Mediterranean Sea. Scientia Marina, 74: 395–410.

SAHRAOUI, I., A. S. HLAILI, H.H. MABROUK, C. LÉGER & S. S. BATES. 2009. Blooms of the diatom genus *Pseudo-nitzschia* H. Pergallo in Bizerte Lagoon (Tunisia, SW Mediterranean). Diatom Research, 24(1): 175-190.

SAHRAOUI, I., S. S. BATES, D. BOUCHOUICHA, H. H. MABROUK & A. S. HLAILI. 2011. Toxicity of *Pseudo-nitzschia* populations from Bizerte Lagoon, Tunisia, southwest Mediterranean, and first report of domoic acid production by *P. brasiliana*. Diatom Research, 26(3): 293-303.

SKEJIĆ, S., N. BOJANIĆ, S. MATIJEVIĆ, O. VIDJAK, B. GRBEC, Ž. NINČEVIĆ GLADAN, S. ŠESTANOVIĆ & D. ŠANTIĆ. 2014. Analysis of

the phytoplankton community in the vicinity of domestic sewage outflow during stratified conditions. Mediterranean Marine Science, 15: 574-586.

STONIK, I. V., T. Y. ORLOVA, I. V. CHIKALOVETS, N. A. AIZDAICHER, A. I. ALEKSANIN, V. A. KACHUR & T. V. MOROZOVA. 2019. *Pseudo-nitzschia* species (Bacillariophyceae) and the domoic acid concentrations in *Pseudo-nitzschia* cultures and bivalves from the northwestern Sea of Japan, Russia. Nova Hedwigia, 108: 73-93.

THESSEN, A. E., H. A. BOWERS & D. K. STOECKER. 2009. Intra- and interspecies differences in growth and toxicity of *Pseudo-nitzschia* while using a different nitrogen sources. Harmful Algae, 8: 792–810.

TRAINER, V. L., S. S. BATES, N. LUNDHOLM, A. E. THESSEN, W. P. COCHLAN, N. G. ADAMS & C. G. TRICK. 2012. *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful Algae, 14: 271-300.

TURK DERMASTIA, T., F. CERINO, D. STANKOVIĆ, J. FRANCÉ, A. RAMŠAK, M. ŽNIDARIČ TUŠEK, A. BERAN, V. NATALI, M. CABRINI & P. MOZETIČ. 2020. Ecological time series and integrative taxonomy unveil seasonality and diversity of the toxic diatom *Pseudo-nitzschia* H. Pergallo in the northern Adriatic Sea. Harmful Algae, 93: doi.org/j.hal.2020.101773.

UJEVIĆ, I., Ž. NINČEVIĆ GLADAN, R. ROJE, S. SKEJIĆ, J. ARAPOV & I. MARASOVIĆ. 2010. Domoic acid-a new toxin in the Croatian Adriatic shellfish toxin profile. Molecules, 15: 6835-6849.

UJEVIĆ, I., R. ROJE- BUSATTO & D. EZGETA-BALIĆ. 2019. Comparison of amnesic, paralytic and lipophilic toxins profiles in cockle (*Acanthocardia tuberculata*) and smooth clam (*Callista chione*) from the central Adriatic Sea (Croatia). Toxicon, 159: 32-37.

VILIČIĆ, D., S. BOSAK, Z. BURIĆ & K. CAPUT-MIHALIĆ. 2007. Phytoplankton seasonality and composition along the coastal NE Adriatic Sea during the extremely low Po River discharge in 2006. Acta Botanica Croatica, 66: 101-115.

WADT, P. R., L. L. MAFRA Jr., C. P. dos SANTOS TAVARES, L. F. FERNANDES & L. A. de OLIVEIRA PROENÇA. 2017. Growth, chain formation, and toxin production by southern Brazilian *Pseudo-nitzschia* isolates under laboratory conditions. Environmental Monitoring and Assessment, 189: 621.

ZINGONE, A., L. ESCALERA, K. ALIGIZAKI, M. FERNÁNDEZ-TEJEDOR, A. ISMAEL, M. MONTRESOR, P. MOZETIČ, S. TAŞ & C. TOTTI. 2020. Toxic marine microalgae and noxious blooms in the Mediterranean Sea: A contribution to the Global HAB Status Report. Harmful Algae, 101843.

Received: 16 June 2020
Accepted: 6 July 2020

Prvi nalaz proizvodnje domoične kiseline u kulturama vrste *Pseudo-nitzschia calliantha* iz srednjeg Jadrana

Jasna ARAPOV, Ivana UJEVIĆ, Maja STRAKA, Sanda SKEJIĆ*, Mia BUŽANČIĆ, Ana BAKRAČ i Živana NINČEVIĆ GLADAN

**Kontakt, e-pošta:* sanda@izor.hr

SAŽETAK

Ovim istraživanjem analizirane su morfološke i toksikološke osobine potencijalno toksičnog roda dijatomeja *Pseudo-nitzschia*. Tri stanične kulture *Pseudo-nitzschia* uspostavljene su iz uzoraka morske vode prikupljenih iz južnog dijela Velebitskog kanala (srednji Jadran) tijekom veljače 2019. Pretražnim elektronskim mikroskopom (SEM) utvrđeno je da sve izolirane kulture pripadaju vrsti *Pseudo-nitzschia calliantha*. Proizvodnja domoične kiseline (DA) potvrđena je za sve analizirane izolate. Najviše koncentracije stanične DA u svim izolatima vrste *P. calliantha* određene su u ranoj uzgojnoj fazi, s najmanjom brojnošću stanica. Ovo istraživanje je prva potvrda proizvodnje DA u kulturama vrste *P. calliantha* izoliranim iz Jadranskog mora.

Ključne riječi: *Pseudo-nitzschia*, morfologija, toksičnost, domoična kiselina